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Organ-specific features of natural killer cells

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Abstract | Natural killer (NK) cells can be swiftly mobilized by danger signals and are among the earliest arrivals at target organs of disease. However, the role of NK cells in mounting inflammatory responses is often complex and sometimes paradoxical. Here, we examine the divergent phenotypic and functional features of NK cells, as deduced largely from experimental mouse models of pathophysiological responses in the liver, mucosal tissues, uterus, pancreas, joints and brain. Moreover, we discuss how organ-specific factors, the local microenvironment and unique cellular interactions may influence the organ-specific properties of NK cells.

Infection and autoimmunity are two common pathological processes, during which inflammation might be elicited in an organ-specific manner. Microorganisms can infect specific organs and induce inflammatory responses. Frequently, the host immune response against the invading pathogens may also cause tissue damage owing to bystander effects. Moreover, inflammation can sometimes trigger organ-specific autoimmune diseases or even malignant transformation of the affected organ.

Among the many cell types of the immune system, natural killer cells (NK cells) are one of the earliest cell types to arrive at target organs of inflammation¹. The role of NK cells in modulating inflammatory responses in different organs is often complex, not always completely understood and sometimes even paradoxical^{2–4}. For example, some studies have shown that NK cells may promote insulinitis, destroy insulin-producing islet cells and promote the development of type 1 diabetes (T1D)^{5–8}. In sharp contrast, other studies have shown that NK cells may inhibit the intensity of inflammatory responses within the central nervous system (CNS)^{9–12}. In rheumatoid arthritis, however, both inhibitory and promoting roles for NK cells have been reported^{13,14}. Although different NK cell subsets or functions might be involved during different stages of the inflammatory process^{2,3}, emerging evidence indicates that additional elements, such as organ-specific factors and cellular interactions, may also influence the divergent functions of NK cells in different organs and during different pathological conditions.

Bone marrow-derived NK cell precursors undergo a maturation process that leads to the acquisition of their effector functions, changes in the expression of chemotactic receptors and adhesion molecules, and their

migration from the bone marrow through the blood to the spleen, liver, lung and many other organs. The distribution of NK cells is not static because these cells can recirculate between organs¹⁵. Owing to the expression of chemokine receptors, such as CC-chemokine receptor 2 (CCR2), CCR5, CXC-chemokine receptor 3 (CXCR3) and CX₃C-chemokine receptor 1 (CX₃CR1), NK cells can respond to a large array of chemokines¹⁶, and thus they can be recruited to distinct sites of inflammation^{17–25} and extravagate to the parenchyma or body cavities. In contrast to B cells, T cells and dendritic cells (DCs), the detailed trafficking patterns of NK cells are not very well characterized. Nevertheless, it appears that chemokines produced by cells that are unique to specific organs may have a role in orchestrating NK cell migration to each organ. For example, the CX₃C-chemokine ligand 1 (CX₃CL1; also known as fractalkine) produced by neurons is necessary and sufficient to guide CX₃CR1-bearing NK cells to the inflamed brain^{10,26}. Similarly, Kupffer cell-derived CC-chemokine ligand 2 (CCL2; also known as MCP1) attracts CCR2-expressing NK cells to the liver during murine cytomegalovirus (MCMV) infection^{27,28}. The production of unique chemokines by organ-specific cell types for the recruitment of NK cells (TABLE 1) suggests that organ-intrinsic elements may be important in shaping NK cell homing during physiological and pathological conditions.

In this article, we discuss recent research on organ-specific NK cells and argue that organ-intrinsic factors shape the cellular, molecular and functional features of NK cells in organ-specific inflammation. The impact of the local environment in tumours on NK cells will not be discussed here because this topic has been extensively covered elsewhere^{29,30}.

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Table 1 | Organ-specific determinants that can influence NK cell function*

Organ-specific determinants	Liver	Mucosal tissues and skin	Uterus	Pancreas	Joints	Brain
Chemokines	Kupffer cell-derived CCL2, CXCL4, CXCL9, CXCL11, CXCL6 and CCL3 (Hs/Mm) ¹⁶	Lung: CCR2, CXCR3 and CX ₃ CR1 ligands (Mm) ¹⁶ Skin: TIG2, CXCR3 ligands and CCR5 ligands (Hs) ⁶⁵	CXCL12 (Hs/Mm) ^{74–82}	CXCL10 (Mm) ⁸⁶	CCR2 and CCR5 ligands (Hs) ^{93,94}	Neuron-derived CX ₃ CL1 and CX ₃ CR1 expression on microglia (Mm) ¹⁰
Unique cellular components	Kupffer cells, hepatic stellate cells, hepatocytes and NKT cells (Hs/Mm) ^{42,52}	Gut: DCs adjacent to cryptopatches; close proximity to epithelial stem cells and Paneth cells (Hs/Mm) ^{67,68} Skin: keratinocytes (Hs) ⁶³	Invading trophoblasts; decidual stromal cells (Hs/Mm) ^{71,72}	β-cells expressing RAE1 (an NKG2D ligand) and ligands for PD1 (Mm) ^{85,88}	Osteoclasts, macrophages and monocytes (Hs/Mm) ^{13,97}	Microglia, astrocytes and neuronal cells (Hs/Mm) ^{1,106,109}
Soluble components	Abundance of IL-12 and IL-18; temporal IL-10 increase; frequent exposure to LPS (Hs/Mm) ^{42,53–55}	Gut: IL-1 and IL-23 derived from DCs (Hs/Mm) ⁶⁸ Lung: IL-15 from lung macrophages (Mm) ⁶⁴ Skin: CCL5 and CXCL10 from keratinocytes (Hs) ⁶⁸	TGFβ derived from stromal cells (Hs) ⁸¹ ; IL-15 (Hs/Mm) ⁷⁴ ; stem cell factor (Mm) ¹⁷	Effector T cell-derived cytokines and T _{Reg} cell-derived cytokines (Mm) ^{6,85}	M-CSF, RANKL, IL-12, IL-15 and IL-18 (Hs/Mm) ^{13,95,97–99}	Neurotransmitters; arrays of cytokines; proteins released by CNS-resident cells (Hs/Mm) ¹
Anatomical constituents	Exposure to food antigens and microbial products; perfusion with blood from the circulation and intestine; thin-walled sinusoids (Hs/Mm) ^{28,42,43}	Gut: exposure to commensal microflora (Hs/Mm) ⁶⁸	Mesometrial lymphoid aggregate of pregnancy (Hs/Mm) ⁷¹	β-cells in the islets of Langerhans (Hs/Mm) ¹²⁷	Relative immune-privilege in normal conditions (Hs/Mm) ¹³	Distinctive blood–brain barrier in healthy conditions; neuro–immune–endocrine axis (Hs/Mm) ^{2,100,128}

CCL, CC-chemokine ligand; CCR, CC-chemokine receptor; CNS, central nervous system; CX₃CL, CX₃C-chemokine ligand; CX₃CR, CX₃C-chemokine receptor; CXCL, CXC-chemokine ligand; CXCR, CXC-chemokine receptor; DC, dendritic cell; IL, interleukin; LPS, lipopolysaccharide; M-CSF, macrophage colony-stimulating factor; NK, natural killer; NKG2D, natural killer group 2, member D; NKT, natural killer T; PD1, programmed cell death protein 1; RAE1, retinoic acid early inducible 1; RANKL, receptor activator of NF-κB ligand; TGFβ, transforming growth factor-β; TIG2, tazarotene-induced gene 2 (also known as chemerin); T_{Reg}, regulatory T. *Hs' indicates evidence found in humans, 'Mm' indicates evidence found in mice, and 'Hs/Mm' indicates evidence found in both humans and mice.

Systemic NK cells

NK cell subsets. NK cells can be divided into functionally distinct subsets, which differ between humans and mice. Human NK cells can be functionally classified based on the levels of CD56 and CD16 expression. Most circulating NK cells are CD56^{low}CD16⁺, and can kill target cells and produce cytokines following specific recognition of their targets^{31,32}. By contrast, CD56^{hi}CD16[–] NK cells, which are the predominant NK cell subset in peripheral lymphoid organs, produce large amounts of cytokines — including interferon-γ (IFNγ), tumour necrosis factor (TNF) and granulocyte–macrophage colony-stimulating factor (GM-CSF) — following stimulation by pro-inflammatory cytokines and acquire cytotoxicity only after prolonged activation^{31,32}. It has been shown that the CD56^{hi}CD16[–] NK cells can differentiate into CD56^{low}CD16⁺ NK cells, and recent studies have revealed that CD56^{low}CD16⁺ NK cells can undergo further differentiation and functional modification with time^{33–35}.

Mouse NK cells share many characteristics with human NK cells, but the lack of CD56 expression and other surface markers on mouse NK cells makes it difficult to identify functionally comparable NK cell populations in mice. Nevertheless, mouse NK cells can be classified based on expression of CD11b (also known as αM integrin), CD27, CD127 and B220 (REFS 36–38). There is evidence that CD11b^{hi}CD27⁺ and CD11b^{hi}CD27[–] NK cell subsets in mice differ in the expression of activating and inhibitory receptors and chemokine receptors, with the CD11b^{hi}CD27⁺ NK cell subset exhibiting the highest levels of both cytokine production and cytotoxicity³⁹. Mouse NK cells that express the interleukin-7 (IL-7) receptor CD127 resemble human CD56^{hi}CD16[–] NK cells in that they produce copious amounts of cytokines and only acquire cytotoxic activities after prolonged activation. Furthermore, B220⁺CD11c⁺ NK cells are enriched in secondary lymphoid tissues and secrete higher levels of IFNγ than other mouse NK cells^{36–38}.

Natural killer cells

(NK cells). Cytotoxic lymphocytes of the innate immune system. These cells have an important role in host defence against many intracellular pathogens and some tumours, regulate the development of adaptive immunity, and control the homeostasis of other immune cells. Human NK cells express the surface marker CD56, and mouse NK cells express the surface markers DX5 (CD49b) and, in some mouse strains (for example, C57BL/6), NK1.1 (CD161).

More recently, Nkp46 (also known as NCR1) has been identified as a common marker for human and murine NK cells. However, some subsets of innate lymphocytes that are distinct from NK cells have also been found to express Nkp46 (BOX 1).

NK cell activation. NK cells are activated by target cells following the detection of missing or altered expression of self MHC class I molecules. NK cell activation can occur through three mechanisms: by engagement of the Fc portion of IgG antibodies that decorate cells; by the sensing of altered molecules on stressed cells; or by an inflammatory environment that is rich in cytokines, such as IL-12 or type I IFNs^{40,41}. Cytotoxicity against infected or transformed cells and production of cytokines are the main effector functions of NK cells.

NK cells in the liver

Homing of NK cells to the liver. The liver represents a unique immunological environment, in which constant exposure to gut-derived antigens does not result in inflammation. In the liver, NK cells are preferentially located in the hepatic sinusoids, often adhering to the endothelial cells⁴². In mice, 5–10% of hepatic lymphocytes are NK cells, whereas, strikingly, up to 30–50% of all human hepatic lymphocytes are NK cells. Moreover, studies in mice have shown that the number of NK cells changes substantially in different experimental models of liver diseases. For example, mice infected with viral pathogens show profound accumulation of NK cells in the liver^{28,43}. Likewise, administration of poly(I:C) (polyinosinic–polycytidylic acid; a synthetic version of double-stranded RNA) also induces NK cell accumulation in the liver. The chemokines CCL2, CCL3 (also known as MIP1α) and CXCL10 (CXCL10), which are produced by liver-resident cells, can recruit NK cells expressing CCR1, CCR2, CCR3, CCR5 and CXCR3 to the liver under various pathological conditions¹⁶.

Hepatic NK cell phenotype. Whereas CD11b⁺CD27⁺ NK cells predominate in the peripheral blood of mice, CD11b⁺CD27⁺ NK cells are found at greater proportions in the liver⁴⁴. Another hallmark of liver NK cells, compared with NK cells in peripheral blood, is the expression of the inhibitory receptor natural killer group 2, member A (NKG2A), which interacts with the MHC class I-related protein Qa-1 in mice (HLA-E in humans). Expression of NKG2A on NK cells may contribute to liver tolerance⁴².

The characteristics of NK cells in different organs may reflect their ability to adapt to different micro-environments. In support of this notion, splenic NK cells can migrate to the liver following adoptive transfer, where they adopt a phenotype and function that closely resembles that of liver-resident NK cells⁴⁴. The recent discovery that NK cells are capable of antigen-specific immunological memory in humans and mice further suggests that NK cells are more versatile than previously anticipated^{45–48}. This is well reflected by hepatic NK cells, as they can acquire and retain antigen-specific memory

Box 1 | Innate lymphoid cells

Recent studies have identified several subsets of innate lymphoid cells (ILCs) that are developmentally related and evolutionarily conserved¹²⁴. This family of cells includes natural killer (NK) cells, lymphoid tissue-inducer (LTi) cells (which produce interleukin-17 (IL-17) and/or IL-22), natural helper cells and nuocytes (which produce IL-5 and IL-13), and cells with characteristics of both NK cells and LTi cells. These latter cells, which have been referred to as NK-like cells or ILC22 cells, share with NK cells the expression of the natural cytotoxicity receptor Nkp46 (but not NK1.1) and with LTi cells the expression of retinoic acid receptor-related orphan receptor-γt (RORγt). However, although NK cells generally produce interferon-γ and are cytotoxic, Nkp46⁺RORγt⁺ ILCs produce IL-22 and are non-cytotoxic. Several subsets of ILCs are enriched in mucosal tissues, particularly in the gut.

NK cells have a crucial role in antiviral immunity; LTi cells are important for the development of lymph nodes during embryogenesis, tissue remodelling, homeostasis of epithelia and host defence; and natural helper cells and nuocytes have been implicated in immunity to extracellular parasites and in the development of asthma. However, the functions of Nkp46⁺RORγt⁺ ILCs remain unclear.

against haptens and virus-derived antigens even in the absence of antigen persistence^{49,50}. Memory NK cells specific for such antigens are enriched in the liver (FIG. 1) and crucially depend on expression of CXCR6, a receptor for the chemokine CXCL16 (which is produced by the endothelium of liver sinusoids⁵⁰). In addition to its role in NK cell-mediated memory, tonic engagement of CXCR6 protects the liver from NK cell-mediated hepatotoxicity by regulating the killing activity of NK cells⁵⁰.

Finally, the chronic stages of hepatic infections with hepatitis C virus (HCV) promote the appearance of NK cells with increased levels of TRAIL (TNF-related apoptosis-inducing ligand), Nkp44 (also known as NCR2), NKG2C and CD122 (REFS 49,50).

Crosstalk between NK cells and liver cells. NK cells may assist in maintaining liver tolerance through their interactions with various cell types. For example, NK cells co-cultured with hepatocytes have been described to promote the capacity of DCs to induce CD4⁺ regulatory T (T_{Reg}) cells⁵¹. The induction of T_{Reg} cells by DCs in the presence of NK cells is dependent on NKG2A engagement on NK cells during their co-culture with hepatocytes⁵².

Kupffer cells, which are mainly localized around the periportal regions in the liver, function as hepatic tissue macrophages and are crucial for endotoxin removal from the blood. Treatment of Kupffer cells with lipopolysaccharide (LPS) results in high levels of IL-10 production, which leads to decreased NK cell activation. It has been suggested that IL-10-mediated induction of NKG2A in liver NK cells might regulate liver NK cell function⁴² (FIG. 1). Although the timing and physiological circumstances under which IL-10 is released by Kupffer cells require further investigation, it is presumed

Type 1 diabetes

A form of diabetes caused by autoimmune destruction of insulin-producing pancreatic β-cells. Autoimmune destruction is predominantly mediated by CD8⁺ T cells, but natural killer cells may affect disease outcome.

Rheumatoid arthritis

A systemic inflammatory disorder that may affect many tissues and organs, but principally attacks synovial joints. The disease process produces an inflammatory response of the synovium (synovitis), secondary to hyperplasia of synovial cells, excess synovial fluid and the development of pannus in the synovium. The pathology of the disease process often leads to the destruction of articular cartilage and ankylosis of the joints.

Kupffer cell

Also known as Browicz–Kupffer cells, Kupffer cells are specialized macrophages that line the walls of the liver sinusoids.

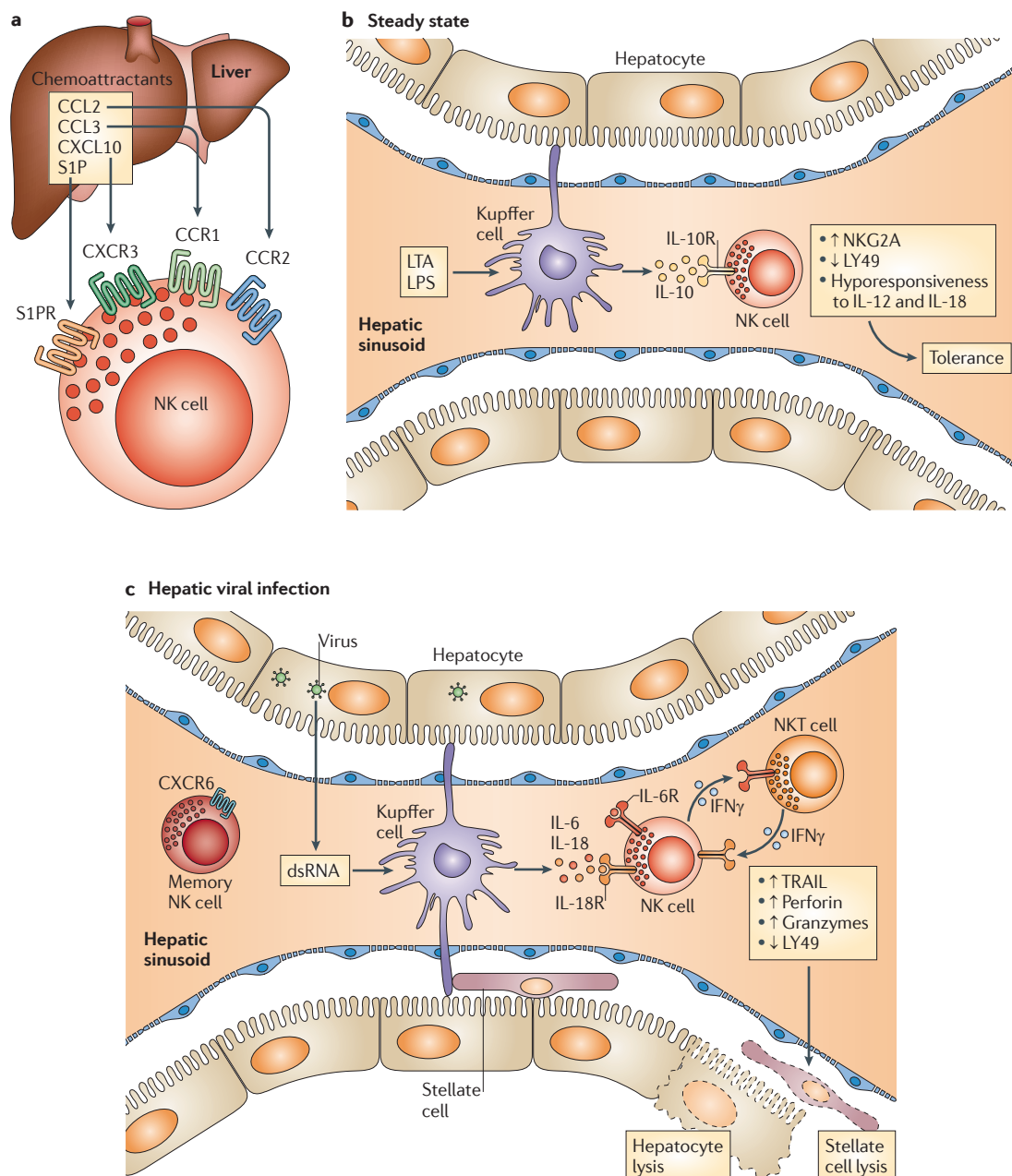


Figure 1 | Unique effector properties of NK cells in the liver. **a** | CC-chemokine ligand 2 (CCL2), CCL3, CXCL10 and sphingosine-1-phosphate (S1P)¹²⁵ derived from Kupffer cells and other hepatic cells attract natural killer (NK) cells to the inflamed liver. **b** | Hepatic NK cells reside in distinctive, thin-walled sinusoids, where Kupffer cells and other lymphocytes are also located. Hepatic NK cells face the challenging task of balancing immunity to multiple hepatotropic viruses and bacteria with tolerance to food and self antigens. In the steady state, commensal-derived lipoteichoic acid (LTA) and lipopolysaccharide (LPS) that arrive in the liver from the gut through the portal vein ligate Toll-like receptor 2 (TLR2) and TLR4, respectively, on Kupffer cells, triggering the release of interleukin-10 (IL-10)¹²⁶. IL-10 in turn renders NK cells hyporesponsive to IL-12 and IL-18 and promotes tolerance in the liver. In addition, expression of the inhibitory receptor natural killer group 2, member A (NKG2A) is increased, whereas expression of the activating receptor LY49 is decreased on NK cells. **c** | During viral infection, double-stranded RNA (dsRNA) ligates TLR3 and triggers Kupffer cells to release IL-6 and IL-18, which in turn activate NK cells and boost their killing activity. This is reflected by the phenotype of hepatic NK cells, which is characterized by the expression of high levels of TNF-related apoptosis-inducing ligand (TRAIL), perforin and granzymes, and a lack of LY49. Activated hepatic NK cells can lyse hepatocytes or stellate cells via TRAIL-dependent pathways during hepatitis B virus infection or fibrosis, respectively. Thus, Kupffer cells appear to have a dual role in shaping hepatic NK cell phenotype and function. Hepatic NK cells are also subjected to the influence of interferon- γ (IFN γ) released by natural killer T (NKT) cells. Hepatic NK cells can acquire memory to antigens derived from influenza virus, vesicular stomatitis virus and HIV-1 (not shown). CCR, CC-chemokine receptor; CXCR, CXC-chemokine receptor; S1PR, S1P receptor.

that Kupffer cells produce IL-10 to downregulate the immune responses orchestrated by NK cells and other immune cells during late stages of inflammation to avoid bystander hepatic damage.

Soluble factors that shape NK cell phenotype in liver. The presence of IFN α , IFN γ , IL-2, IL-6, IL-12 and IL-18 in the inflamed liver largely contributes to an activated phenotype of NK cells, whereas IL-15 and IL-2 may sustain the survival and activation of these cells (FIG. 1). Among the liver-resident cells that can provide a source of these cytokines, hepatic natural killer T cells (NKT cells) can release large amounts of IFN γ , and Kupffer cells primarily release CCL2, IL-6 and other acute pro-inflammatory mediators (FIG. 1). As mentioned above, Kupffer cells can also release IL-10 (REF. 53), which induces expression of NKG2A⁵¹. The liver encounters relatively high levels of LPS derived from the gut, which could be potentially harmful. Interestingly, it has been reported that crosstalk between Toll-like receptor (TLR) and complement receptor signalling pathways inhibits IL-12 production, and this may have a role in dampening excessive NK cell activation^{54,55}.

Role of hepatic NK cells in disease. Chronic liver disease is often associated with the development of fibrosis, a wound-healing process. Fibrosis is characterized by the formation of new blood vessels, sinusoidal remodelling and pericyte (stellate cell) proliferation⁵⁶. Hepatic stellate cells are activated following the phagocytosis of apoptotic bodies and cell debris, leading to their differentiation, proliferation and pro-fibrotic progression. NK cells have been suggested to be involved in hepatic stellate cell death, as depletion of NK cells leads to enhanced fibrosis in a mouse experimental model⁴². Interestingly, of the many pro-apoptotic molecules investigated in activated hepatic stellate cells, the upregulation of TRAIL receptors, which can bind NK cell-derived cytotoxic ligands may be crucial⁵⁷.

The finding that NKG2A expression is increased on NK cells from patients chronically infected with HCV suggests a role for these cells in maintaining the persistent viral infection^{58,59}, possibly by inhibiting antiviral T cell responses and facilitating pathogen persistence. Other studies have suggested that hepatic NK cells may contribute to innate resistance to HCV infection via increased expression of Nkp30 (also known as NCR3)⁶⁰. Finally, liver NK cells might contribute to the therapeutic activities of IFN α against hepatitis B virus (HBV) and HCV infection. In addition to its direct antiviral effects, IFN α treatment might affect NK cell activity⁶¹. Transcriptional profiling studies using gene arrays identified *TRAIL* as one of the most significantly upregulated genes in NK cells after IFN α stimulation. IFN α -stimulated NK cells killed HCV-infected hepatoma cells in a TRAIL-dependent manner. These findings suggest that IFN α -induced TRAIL expression on NK cells may be associated with control of HCV infection⁶¹.

Thus, steady-state hepatic NK cells promote liver tolerance and contain a population with memory-like features. Following viral infection, chemokines that are produced by liver-resident cells recruit more NK cells to

the liver, and these cells acquire an activated phenotype to control viral infection. Crosstalk with liver-resident cells restrains NK cell-mediated cytotoxicity following viral clearance to avoid bystander tissue damage. However, during chronic liver infections, NK cells promote the persistence of the pathogen and liver injury while inhibiting liver fibrosis and tissue regeneration.

NK cells in mucosal tissues and the skin

NK cell homing to the lung, skin and gut. Interpretation of studies performed with NK cells in mucosal tissues is complicated by the fact that many studies often have not been able to distinguish bona fide NK cells from NKT cells and the growing population of innate lymphoid cells (ILCs) that are particularly prevalent in the gut-associated lymphoid tissue (GALT) (BOX 1). Nevertheless, based on the available evidence, NK cells represent a major lymphocyte subset in the healthy lung (accounting for ~10% of all lymphocytes in the lung in mice)¹⁶, and are also present in healthy skin in humans⁶² and in the GALT of humans and mice⁶⁵.

In humans, infection with *Staphylococcus aureus* triggers a vigorous NK cell influx into the broncho-alveolar space⁶⁴. Ligand of diverse chemokine receptors, including CCR2, CXCR3 and CX₃CR1, with their corresponding ligands recruits NK cells to the lung during various pathogen encounters¹⁶.

In acute skin lesions, such as psoriatic plaques in human patients, 5–8% of the infiltrating cells are CD3⁺CD56⁺ NK cells, which are mostly localized in the mid and papillary dermis⁶³. Skin NK cells express high levels of the chemokine receptors CXCR3 and CCR5, intermediate amounts of CXCR1, CCR6, chemokine-like receptor 1 (also known as CHEMR23) and CCR8, and low levels of CCR1, CCR2, CCR4, CCR7 and CX₃CR1 (REF. 65). Moreover, they home to the skin, directed by CXCL10 and CCL5 (REF. 63) (TABLE 1).

In the intestine, NK cells are found predominantly within the lamina propria and are rare in lymphoid aggregates, although they can be found in the para-follicular region of caecal lymphoid patches, Peyer's patches and mesenteric lymph nodes⁶⁵.

NK cell phenotype in mucosal tissues and the skin.

NK cells generated *in vitro* from lung NK cell progenitors differ substantially from NK cells generated from bone marrow-derived NK cell progenitors with regard to expression of members of the LY49 family of NK cell receptors. Specifically, lung NK cells express multiple LY49 receptors, whereas most NK cells that develop from bone marrow-derived progenitors express CD94–NKG2 heterodimers but not LY49, suggesting a major impact of the microenvironment on NK cell development⁶⁶. During *S. aureus* infection, lung NK cells produce increased amounts of TNF⁶⁴.

In addition to their distinct patterns of chemokine receptor expression, NK cells in the skin of patients with psoriatic lesions exhibit a CD56^{hi}CD16⁺CD158b⁺ phenotype, fail to express the skin-homing cutaneous lymphocyte-associated antigen and release abundant IFN γ following stimulation⁶³.

Natural killer T cells
(NKT cells). A subset of T cells that are specific for lipid or glycolipid antigens bound to the MHC class I-related protein CD1d. Most NKT cells, which are referred to as type I or invariant NKT (iNKT) cells, express an invariant T cell receptor- α chain (Va14–Ja18 in mice or Va24–Ja18 in humans) together with various natural killer cell surface markers, such as NK1.1. NKT cells have a regulatory role during an immune response, bridging the innate and adaptive immune systems. iNKT cells can be most easily identified with multimeric CD1d molecules loaded with iNKT cell agonists, such as α -galactosylceramide.

A major challenge in identifying gut NK cells lies in the difficulty in discriminating these cells from other gut ILCs. A subset of gut ILCs expresses NKp46, and among this subset one fraction lacks expression of retinoic acid receptor-related orphan receptor- γ t (ROR γ t) and produces IFN γ ⁶⁷. Among gut ILCs, these NKp46⁺ROR γ t⁺ cells resemble conventional NK cells in several respects, such as in the expression of several NK cell receptors and various molecules that are required for NK cell-dependent cytotoxicity⁶³, and thus these cells are likely to be bona fide gut NK cells. However, adding to the complexity is the observation that, whereas ROR γ t expression is stable in 'non-NK' NKp46⁺ROR γ t⁺ cells in the small intestine, ROR γ t expression can be lost in these cells in the colon, leading to a population of IFN γ -polarized NKp46⁺ROR γ t⁺ cells that are phenotypically indistinguishable from gut NK cells⁶⁷.

Crosstalk between NK cells and mucosal tissue- or skin-resident cells. In the lung, alveolar macrophage-derived IL-15 supports NK cell proliferation and, conversely, lung macrophages show improved phagocytosis of *S. aureus* in the presence of NK cells⁶⁴. Similarly, there appears to be a reciprocal relationship between skin-infiltrating NK cells and keratinocytes. Keratinocytes may provide a major source of CCL5 and CXCL10 to recruit NK cells, and skin-resident NK cells release large amounts of IFN γ and efficiently activate keratinocytes⁶³. In the gut, signals emanating from DCs, epithelial cells and luminal contents constantly regulate GALT development and function, thereby contributing to the maintenance of a 'tolerogenic' status to innocuous antigens and commensal flora while initiating immune responses and tissue reparative mechanisms that restrain pathogen invasion⁶⁷. For example, IL-23 enhances the production of IL-22 by NKp46⁺ROR γ t⁺ cells^{67,68}, and this may function to preserve and restore epithelial barrier integrity⁶⁷.

Role of mucosal NK cells in disease. NK cells, including those in the gut and other mucosal lymphoid organs, are thought to protect against infection by viruses and other intracellular pathogens. For example, within 24 hours of infection with orally administered *Listeria monocytogenes*, IFN γ production is induced by NK cells in the small intestine⁶⁹. IFN γ production peaks at 48 hours post-inoculation in this model. Importantly, blocking IFN γ , but not IL-22, with neutralizing antibodies induces significant increases in bacterial loads in the GALT⁶⁹. However, this does not exclude an important role for IL-22 in other bacterial infections or in other forms of tissue injury^{69,70}.

In summary, NK cells are detected in healthy mucosal tissues and the skin. In response to infection, more NK cells are recruited to the lung, the gut and the skin, and these cells acquire an activated phenotype, which is essential for pathogen clearance. Moreover, in the gut, classical NK cells together with ILCs appear to control inflammatory responses.

NK cells in the uterus

NK cell homing to the uterus. NK cells are abundant in the decidua — the endometrium of the pregnant uterus that forms the maternal part of the placenta.

There, uterine NK cells are thought to control endometrial tissue remodelling, vascular function and the formation of a placenta in the uterus^{71,72}. Several studies have described the kinetics of uterine NK cell accumulation in pregnant mice^{72,73}. Following fertilization and implantation, NK cells accumulate initially in the decidua basalis, which is subjacent to the trophoblast layer, and later in the mesometrial lymphoid aggregate of pregnancy, a lymphoid structure that transiently forms between the two layers of myometrial smooth muscle. Uterine NK cell numbers peak at mid-gestation (9–10 days) and decline in the last 5 days of pregnancy⁷³. Uterine NK cells can either develop *in situ* or migrate from the peripheral blood through a CXCR4- and CXCL12-dependent mechanism (reviewed in REF. 74) (TABLE 1).

Uterine NK cell phenotype. During pregnancy, the human uterine decidua is dominated by the presence of CD56^{hi}CD16⁺ NK cells⁷⁵. In the mouse, two distinct subsets of CD3⁺CD122⁺ NK cells have been described in the uterus at mid-gestation⁷⁶ (TABLE 2). The smaller subset expresses phenotypic markers of peripheral NK cells and might therefore represent peripheral NK cells that have recently been recruited into the uterus. The larger subset lacks NK1.1 expression and reacts with *Dolichus biflorus* agglutinin, which specifically identifies uterine NK cells in the mouse. Uterine NK cells express a unique repertoire of MHC class I-binding receptors, activation markers and adhesion molecules. The unique LY49 receptor repertoire of mouse uterine NK cells suggests that they develop within the uterus and that the local microenvironment may shape the LY49 receptor repertoire⁷⁶. Similarly, NK cells in the human uterine mucosa have recently been characterized and found to resemble unique early developmental stages of human NK cell differentiation⁷⁷.

Crosstalk between uterine NK cells and trophoblasts during pregnancy. The function of uterine NK cells is still not clear, but it appears that they interact with invading trophoblasts, and this interaction results in the release of factors that modify the uterine spiral arteries. This 'physiological' inflammatory process is important as it increases the blood supply to the mouse fetus^{71,78,79}. Moreover, uterine NK cell-derived IFN γ is needed for vascular modifications to occur during pregnancy⁷⁸. Although mice lacking uterine NK cells or components of the IFN γ pathway do not exhibit increased fetal loss, they show several abnormalities during pregnancy, including reduced decidual cellularity and inadequate modifications of the spiral arteries⁷³.

In addition, the regulation of NK cell activation is crucial for normal placentation and hence a successful pregnancy. Interestingly, human immunogenetic studies have suggested that interactions between fetal HLA-C molecules and killer cell immunoglobulin-like receptors (KIRs) on uterine NK cells are important for reproductive success in humans, possibly through tuning the threshold for NK cell activation⁸⁰.

Trophoblast layer

A layer formed by trophoblast cells that arise from the trophoblast that surrounds the blastocyst. The trophoblast layer attaches the embryo to the uterus and forms a part of the placenta.

Table 2 | Unique features of NK cells in distinct organs*

Cell features and functions	Liver	Mucosal tissues and skin	Uterus	Pancreas	Joints	Brain
Receptor expression	NKG2A ↑, LY49 ↑, CD56 ^{low} and CD16 ↓ (Hs/Mm) ^{44,49,50}	Gut: RORγt ⁺ LIN ⁻ , RORγt ⁺ NKp46 ⁺ NK1.1 ^{int} , CD127 ^{int} c-Kit ⁺ and KIR ⁻ (Hs) ^{65,67-69} ; LY49 ⁻ (Mm) ⁶⁸ Lung: LY49 ^{hi} (Mm) ^{64,66} Skin: CD56 ^{hi} CD16 ⁻ CD158b ⁻ (Hs) ⁶³	CD16 ⁻ CD56 ^{hi} (Hs) ⁷⁵ ; NKp46 ⁺ (Hs/Mm) ⁷⁵ ; CD3 ⁺ CD122 ⁺ , LY49 ⁺ , NK1.1 ⁺ and DX5 ⁻ (Mm) ^{76,79}	KLRG1 ↑; NK cells from NOD mice express more NKC gene products than NK cells from B6.H-2 ^{g7} mice; CD25 and CD69 (Mm) ^{6,85}	CD56 ^{hi} CD16 ⁻ ↑, CD94–NKG2A ↑ (Hs) ^{87,92,95,96} ; RANKL ⁺ , chemokine receptors ↑ (Hs/Mm) ^{13,99} ; M-CSFR ⁺ , adhesion molecules ↑ (Mm) ^{13,97,98}	NKG2A ↑ (Mm) ^{11,12} ; nicotinic acetylcholine receptors ↑ (Mm) [†]
Effector molecule expression	NKp44 ⁺ , NKG2C ⁺ , TRAIL ↑, perforin and granzymes ↑ (Mm) ^{44,49,50}	Gut: IL-22 ⁺ , IL-17 ⁺ , IFNγ ⁻ and perforin ↓ (Hs/Mm) ⁶⁷ Lung: factors that enhance macrophage function (Mm) ⁶⁴ Skin: IFNγ (Hs) ⁶³	CXCL8 and CXCL12 ↑ (Hs) ⁸² ; IFNγ, VEGF and PLGF ↑ (Mm) ^{74,78}	PD1 ⁺ and LAMP1 ⁺ (Mm) ^{85,88}	Granzymes and IFNγ (Hs/Mm) ^{13,87,92}	IFNγ, perforin and granzymes ↑ (Mm) ^{11,12}
Cytotoxicity and proliferation	Increased early, decreased at later stages (Hs/Mm) ⁴²	Gut: reduced or absent (Hs/Mm) ^{67,68}	ADCC and cytotoxicity decreased (Hs) ⁸¹	Proliferation and degranulation increased early and decreased at later stages (Mm) ⁸⁵	Increased (Mm) ¹³	Increased cytotoxicity against microglia (Mm) ^{11,12}
Other functions	Crosstalk with Kupffer and NKT cells (Hs/Mm) ⁴² ; promote T _{Reg} cells (Mm) ⁵¹	Control inflammation (Hs/Mm) ^{67,84} Gut: T _H 17 cell-like functions; respond to DC-derived IL-23 and IL-1, but not IL-15; sustain lymphoid tissue integrity; promote tissue repair (Hs/Mm) ^{67,68}	Regulate endometrial remodelling, angiogenesis and implantation (Hs/Mm) ⁷¹ ; uterine NK cell-derived IFNγ modifies vascularity (Mm) ⁷⁸	Reciprocal interactions with effector and T _{Reg} cells (Mm) ⁶	Promote formation of osteoclasts; colocalize with monocytes and promote their TNF production (Mm) ¹³	Condition the CNS; release cytokines; suppress myelin-specific T _H 17 cells (Mm) ^{11,12}

ADCC, antibody-dependent cell-mediated cytotoxicity; CNS, central nervous system; CXCL, CXC-chemokine ligand; DC, dendritic cell; IFNγ, interferon-γ; IL, interleukin; KLRG1, killer cell lectin-like receptor subfamily G member 1; LAMP1, lysosomal-associated membrane protein; LIN⁻, lineage negative; M-CSF, macrophage colony-stimulating factor; NK, natural killer; NKC, natural killer gene complex; NKG2, natural killer group 2; NKT, natural killer T; NOD, non-obese diabetic; PLGF, placental growth factor; RANKL, receptor activator of NF-κB ligand; T_H17, T helper 17; TNF, tumour necrosis factor; TRAIL, TNF-related apoptosis-inducing ligand; T_{Reg}, regulatory T; VEGF, vascular endothelial growth factor. *Hs indicates evidence found in humans, 'Mm' indicates evidence found in mice, and 'Hs/Mm' indicates evidence found in both humans and mice. †Unpublished observations (F.-D.S.).

Soluble factors that shape NK cell phenotype in the uterus. Transforming growth factor-β (TGFβ) produced by decidual stromal cells can reduce CD16 expression on NK cells, thereby impairing antibody-dependent cellular cytotoxicity (ADCC)⁸¹. CXCL12 is produced in the decidua, particularly by extravillous trophoblasts, and attracts peripheral NK cells towards the decidua⁸²; on the other hand, the presence of IL-15 and stem cell factors may support NK cell development *in situ*⁷⁴ (TABLE 1).

Role of uterine NK cells in pregnancy disorders. Uterine NK cell activation following interaction with invading trophoblasts has been associated with several pregnancy disorders. A seminal study described how a particular combination of fetal HLA-C molecules and maternal inhibitory NK cell receptors was associated with a predisposition for pre-eclampsia⁸³. More recently, such studies have been extended to recurrent miscarriage and fetal growth restriction, revealing a common uterine NK cell-mediated mechanism underlying these pregnancy disorders⁸⁴. Importantly, this study also showed that mothers with uterine NK cells that express activating and inhibitory NK cell

receptors with similar specificities are less likely to suffer from unsuccessful pregnancy. Although data from human population genetics studies have provided interesting insights into the factors controlling interactions between uterine NK cells and trophoblasts of fetal origin, functional studies in humans are challenging. Thus, despite differences in placentation between humans and mice⁷¹, mice may provide an important model for studying the biology of uterine NK cells.

In summary, uterine NK cells can either develop *in situ* or migrate to the decidua after being attracted by locally produced chemokines. Their receptor repertoire suggests that their activation has to be tightly regulated to ensure normal pregnancy. Moreover, mouse and human studies suggest that crosstalk between uterine NK cells and trophoblasts contributes to vascular modifications during pregnancy.

NK cells in the pancreas

NK cell homing to the pancreas. NK cells are present not only in the pancreas of diabetes-prone non-obese diabetic (NOD) mice but also in the pancreas of mouse strains that are not susceptible to the development of

diabetes⁸⁵. This indicates that NK cells can be recruited to the pancreas in the absence of profound inflammation. Nevertheless, the severity of T1D in NOD mice correlates with increased numbers and early entry of NK cells into the pancreas^{5,6,85}, suggesting a disease-promoting role for these infiltrating NK cells. Importantly, one study showed that NK cells accumulate before T cells in the islet infiltrates⁸⁵. NK cells might be recruited to the pancreas by chemokines such as CXCL10 (a ligand for CXCR3 on NK cells) and CCL5 (a ligand for CCR5 on NK cells), which can be produced by multiple cell types, including pancreatic β -cells (TABLE 1), and their numbers are increased in the pancreas of NOD mice in an accelerated model of T1D⁸⁶.

Pancreatic NK cell phenotype. Molecular, phenotypic and functional characteristics of NK cells in the pancreas and spleen have been compared between diabetes-prone NOD mice and diabetes-resistant mice^{5,85}. Microarray studies revealed that the NK cell gene complex, which encodes a set of NK cell receptor genes, is expressed differently in diabetes-prone NOD mice versus diabetes-resistant B6.H-2⁸⁷ mice (which are congenic for the NOD MHC region). Immune phenotyping analyses further indicated that pancreatic NK cells from NOD mice share phenotypic characteristics with pancreatic NK cells from non-diabetic strains, but display increased levels of the activation markers CD69 and CD25, and of surface killer cell lectin-like receptor G1 (KLRG1), which is a marker for mature NK cells that have undergone proliferation⁸⁵. Furthermore, a subset of pancreatic NK cells from young NOD mice produced IFN γ and expressed the lysosomal marker lysosome-associated membrane protein 1 (LAMP1; also known as CD107a)⁸⁵, which is a marker for NK cell degranulation (TABLE 2).

However, proliferating and activated NK cells were detected only in the early pre-diabetic infiltrates⁸⁵. During the later stages of diabetes development, most pancreatic NK cells of NOD animals became hyporesponsive, as reflected by diminished cytokine secretion and a lower capacity to degranulate in response to antibodies specific for activating receptors, and in response to activation by phorbol 12-myristate 13-acetate (PMA) and ionomycin. It was thus proposed that pancreatic NK cells from NOD mice initially mediate pro-inflammatory effector functions, but later become hyporesponsive owing to exhaustion or regulation⁸⁵.

Crosstalk between NK cells and β -cells. Experiments in which splenic NK cells from NOD mice were transferred into recombination activating gene (RAG)- and common cytokine receptor γ -chain (γ_c)-deficient NOD mice (which lack B cells, T cells and NK cells) showed that NK cells migrate to the pancreas and upregulate CD69 and KLRG1 expression⁸⁵. This suggests that the local micro-environment in the pancreas of NOD mice, in the absence of profound inflammation and adaptive immune cells, can recruit NK cells and regulate the phenotype of these cells. In addition, one study suggested that the NK cell activating receptor Nkp46 can recognize ligands on β -cells that promote NK cell activation, which, in turn, might result in

β -cell destruction⁸. Such activated NK cells might exhibit direct cytotoxicity against pancreatic β -cells and recruit other cell types, and thus have a pathogenic role during the early stages of diabetes development.

The hyporesponsive phenotype of NK cells that is observed during later stages of diabetes development might be partly caused by interactions between NK cell receptors and their ligands expressed by pancreatic β -cells. For example, it has been suggested that ligands for NKG2D — such as retinoic acid early inducible 1 (RAE1; also known as mRNA export factor) expressed by pancreatic β -cells — might downregulate the expression of NKG2D and ‘turn off’ NK cells to avoid excessive autoimmunity⁸⁷. Furthermore, interaction of the inhibitory receptor programmed cell death protein 1 (PD1) on pancreatic NK cells with PD1 ligands on inflamed islets might dampen the response of pancreatic NK cells^{85,88}.

Soluble factors that shape NK cell phenotype in the pancreas. The phenotype of NK cells in the pancreas might be influenced by cytokines produced by other cell types. For example, it has been suggested that cytokines derived from autoreactive T cells might contribute to the hyporesponsive phenotype of NK cells during the later stages of diabetes development in NOD mice⁸⁷. There is also evidence that NK cell functions are kept in check by T_{Reg} cells, as ablation of CD4⁺FOXP3⁺ T_{Reg} cells in diabetes-prone BDC2.5 T cell receptor (TCR)-transgenic NOD mice results in rapid activation of NK cells within the insulinitic lesions, before the activation of effector T cells⁶.

Role of pancreatic NK cells in disease. A pathogenic role of NK cells in T1D has been supported by evidence derived from several animal models^{5–8,86,89}. Depletion of NK cells significantly inhibited diabetes development in three mouse models: BDC2.5 TCR-transgenic NOD mice treated with cytotoxic T lymphocyte antigen 4 (CTLA4)-specific antibodies⁵; transgenic NOD mice with pancreatic expression of suppressor of cytokine signalling 1 (SOCS1) that had been infected with coxsackievirus B4 (REF. 89); and transgenic NOD mice with β -cell-specific expression of IFN β ⁸⁶. Furthermore, blockade of the NK cell activating receptor NKG2D prevented disease in NOD mice⁷, although the contribution of other NKG2D-expressing cells (including CD8⁺ T cells) could not be ruled out in this model.

In addition to their pathogenic roles in autoimmune diabetes, NK cells can, paradoxically, exhibit protective functions in β -cell autoimmunity. NK cells have a crucial role in mediating the capacity of complete Freund's adjuvant (CFA) to protect NOD mice from diabetes⁹⁰. NK cells also have an important role in islet allograft tolerance in C57BL/6 mice induced by blocking interactions between CD40 and CD154 or between lymphocyte function-associated antigen 1 (LFA1; also known as α L β 2 integrin) and intercellular adhesion molecule (ICAM)⁹¹. It is likely, however, that these ameliorating effects of NK cells on autoimmunity and allograft rejection are mediated by peripheral rather than pancreatic NK cells, possibly through them interacting directly with DCs or pathogenic T cells.

In summary, few NK cells home to the healthy pancreas, and the numbers of pancreatic NK cells are substantially increased in diabetes-prone and diabetic mice. The local microenvironment of the pre-diabetic pancreas seems to induce NK cell proliferation and activation. At later disease stages, crosstalk between pancreatic NK cells and β -cells or other immune cells leads to NK cell hyporesponsiveness. Despite their early pathogenic role in diabetes, NK cells have also been implicated in CFA-mediated diabetes protection and in islet allograft tolerance.

NK cells in the joints

NK cell homing to the joints. Whereas few NK cells are present in healthy joints, these cells are abundant in the inflamed joints of patients with rheumatoid arthritis⁹² and in mice with collagen-induced arthritis¹³. NK cells can be detected in the inflamed synovial tissue at any stage of the disease, and they constitute up to 20% of all lymphocytes in synovial fluid of patients with established rheumatoid arthritis⁹². These cells express increased levels of the chemokine receptor CCR5 and, to a lesser extent, CCR2 (REF. 93). Chemokine ligands for these receptors have been detected in the synovial fluid of patients with rheumatoid arthritis⁹⁴ (TABLE 1).

NK cell phenotype in the joints. Among synovial fluid NK cells in patients with rheumatoid arthritis, cells of the CD56^{hi}CD16⁻ phenotype predominate^{92,95,96}, suggesting that this cell population is selectively recruited to the inflamed synovium and/or expands there. These cells also express high levels of the inhibitory receptor NKG2A, produce granzymes and exhibit enhanced cytotoxicity compared with systemic NK cells^{87,92} (TABLE 2). Uniquely, NK cells in the joints of mice with collagen-induced arthritis express receptor activator of NF- κ B ligand (RANKL) and macrophage colony-stimulating factor (M-CSF)^{97,98}, a feature that is shared with NK cells from patients with rheumatoid arthritis⁹⁹.

Crosstalk between NK cells and other cells in inflamed joints. The molecular features of synovial fluid NK cells and their interaction with other cells in the inflamed joint have been investigated in animal models. NK cells in the inflamed joint that express RANKL and M-CSF are frequently juxtaposed with monocytes in the synovium of inflamed joints¹³ (TABLE 2). The reciprocal interactions between these two cell types can trigger the formation of osteoclasts in a process that is dependent on RANKL and M-CSF¹³.

Soluble factors that shape NK cell phenotype in joints. Cytokines, such as IL-12, IL-15 and IL-18, that are produced by myeloid cells in the inflamed joint can stimulate IFN γ production by NK cells⁹⁵. These factors, in particular IL-15, might also contribute to the induction of RANKL and M-CSF in synovial NK cells¹³.

Role of joint NK cells in disease. Depletion of NK cells from mice before the induction of collagen-induced arthritis reduces the severity of subsequent arthritis and almost completely prevents bone erosion¹³.

Paradoxically, however, NK cells were shown to have a crucial role in protection against inflammatory arthritis mediated by unmethylated CpG oligodeoxynucleotides following transfer of serum from K/BxN mice into wild-type mice¹⁴. This protection required interaction of NK cells with DCs in lymphoid organs, resulting in reduced neutrophil recruitment to the joints. These observations suggest opposite functions of NK cells in lymph nodes versus inflamed joints during rheumatoid arthritis. The current available information is not sufficient to provide a clear linkage between the divergent phenotypes and functions of NK cells in lymphoid organs and joints. Differences in the triggering antigens (that is, type II collagen versus glucose-6-phosphate isomerase) in different models of arthritis and their roles in inducing NK cells directly or indirectly invite further investigation.

Thus, NK cells are recruited to the inflamed joints, where they exhibit an activated phenotype and promote osteoclast differentiation and inflammation. By contrast, lymph node NK cells have been suggested to play a crucial part in CpG-induced protection against inflammatory arthritis in a mouse disease model.

NK cells in the CNS

Homing of NK cells to the CNS. In the CNS, the brain and spinal cord are shielded from the circulating blood by the blood–brain barrier and the epithelial blood–cerebrospinal fluid barrier, respectively, which are formed by the tight junctions of endothelial cells and prevent the influx of immune cells under normal physiological conditions. This view was recently challenged by the finding that activated T cells can breach the blood–brain barrier and the epithelial blood–cerebrospinal fluid barrier to perform immune surveillance functions in the CNS¹⁰⁰. It is conceivable that peripherally activated lymphocytes, including NK cells, might also be able to penetrate the blood–brain barrier. However, data on the presence of NK cells in the human brain and spinal cord are sparse. Nevertheless, NK cells can be detected in mouse CNS tissues during a variety of infections, including infection by murine coronavirus¹⁰¹, Semliki Forest virus¹⁰² and *L. monocytogenes*¹⁰³. NK cells are also recruited to the CNS in glioma-bearing mice; 1 week after tumour implantation, NK cells constitute approximately 50% of all leukocytes in the CNS, and after this peak their frequency gradually declines¹⁰⁴. NK cells might be recruited to the CNS by chemokines such as CX₃CL1 produced by neurons and CCL2 and CXCL10 produced by microglia, astrocytes or infiltrating inflammatory cells (TABLE 1).

NK cell phenotype in the CNS. The most intriguing data on the role for NK cells in CNS inflammation are derived from studies of experimental autoimmune encephalomyelitis (EAE). In this model, NK cells become fully activated and proliferate in the CNS even before the arrival of T cells¹⁰⁵. Analysis of NK cells in the CNS of mice with EAE suggested that, compared with their peripheral counterparts, CNS-infiltrating NK cells upregulate the inhibitory receptor NKG2A and produce large amounts of CCL2, a chemoattractant for microglia^{11,12}.

Collagen-induced arthritis
A model for human rheumatoid arthritis that is induced in susceptible strains of mice or rats by immunization with autologous or heterologous type II collagen in adjuvant.

K/BxN mice
K/BxN mice express a transgene-encoded T cell receptor that is specific for the ubiquitously expressed enzyme glucose-6-phosphate isomerase, which is presented by the MHC class II molecule H2-A⁹⁷. These mice spontaneously develop joint inflammation with features of human rheumatoid arthritis. Interestingly, transfer of serum from arthritic animals into healthy non-transgenic animals induces arthritis in a highly reproducible and controlled manner.

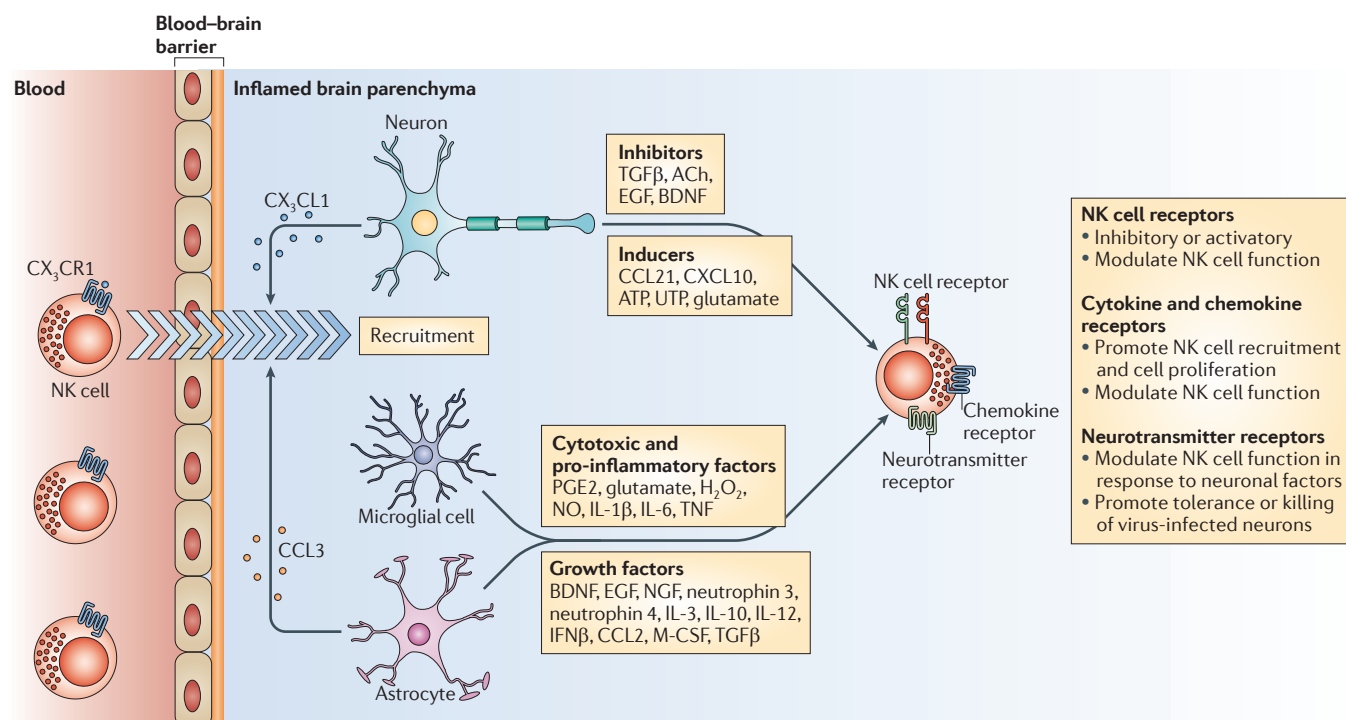


Figure 2 | The brain alters NK cell phenotypes and functions. The entry of lymphocytes into the central nervous system (CNS) is normally shielded by the blood–brain barrier, which becomes compromised in several pathological circumstances. Natural killer (NK) cells are recruited to the inflamed brain by CX₃C-chemokine ligand 1 (CX₃CL1) produced by neurons. Brain-specific cell types and neurotransmitters alter the features of NK cells that migrate from the periphery. An array of cytokines, growth factors and neurotransmitters produced by microglial cells, astrocytes and neurons can influence NK cell activation and proliferation. The chemokine CC-chemokine ligand 3 (CCL3) attracts NK cells to the site of inflammatory foci. After acquiring new features, brain-resident NK cells lose tolerance to microglial cells, suppress pathogenic, myelin-reactive T helper cells and significantly attenuate the intensity of local inflammatory and autoimmune responses. NK cells also kill neurons infected by herpes simplex virus. In addition, mediators derived from neurons, microglial cells and astrocytes and multidirectional cellular interactions may influence NK cell tolerance and function. The combined effects of soluble factors and cellular interactions, as well as the timing of the immune response, may dictate NK cell activity in the CNS. ACh, acetylcholine; BDNF, brain-derived neurotrophic factor; CXCL10, CXC-chemokine ligand 10; EGF, epidermal growth factor; IFN β , interferon- β ; IL, interleukin; M-CSF, macrophage colony-stimulating factor; NGF, nerve growth factor; NO, nitric oxide; PGE₂, prostaglandin E₂; TGF β , transforming growth factor- β ; TNF, tumour necrosis factor.

Microglia

Non-neuronal cells of the central nervous system with phagocytic properties. They are haematopoietic cells and are involved in inflammatory and immune responses in the central nervous system.

Experimental autoimmune encephalomyelitis

(EAE). An experimental model for human multiple sclerosis that can be induced in susceptible animals by immunization with neuroantigens and adjuvant.

Crosstalk between NK cells and CNS-specific cell types. The CNS is distinguished from other organs with regard to the genesis and progression of inflammatory responses in that it harbours a unique spectrum of antigen-presenting cells (APCs; that is, astrocytes and microglia) and neuroantigens, and accommodates intimate interactions between the immune and nervous systems (FIG. 2). So, after homing to the inflamed CNS, NK cells become receptive to an array of cellular components that they have not encountered in the periphery. These include astrocytes, microglia and neurons^{106–109}, which release numerous soluble factors with diversified and perhaps coordinated effects on NK cells (FIG. 2).

The finding that NK cells in the CNS control the magnitude of an inflammatory response in the EAE model^{11,12} suggests that, after homing to the CNS, NK cells exert specific functions that affect the outcome of inflammation. In particular, it has been suggested that NK cells in the CNS can directly lyse microglia^{11,12}.

Microglia are macrophage-like APCs that are capable of priming myelin-reactive T cells, and they exert direct effects on neuronal or glial cells depending on the timing of the inflammatory process and the activation status of these cells^{105–111}. Microglia serve as sentinels for ongoing immune responses in the periphery that have the potential to spread to the CNS. In comparison with the behaviour of other types of APC in the CNS during brain inflammation, activated microglia appear to become targets of peripherally activated NK cells that enter the CNS¹¹. Such interactions between NK cells and microglia are likely to be facilitated by the recruitment of microglia through CCL2 secretion by NK cells. As activated microglia have a crucial role in initiating and propagating pathogenic T cell responses during EAE, their elimination results in reduced numbers of myelin-reactive T helper 17 (T_H17) cells in the CNS¹¹. Although the exact mechanisms remain unclear, susceptibility of activated microglia to NK cell lysis may occur through the

loss of MHC class I expression on these cells^{11,12} (FIG. 2). These findings in mice are in apparent contrast with a recent study suggesting that human NK cells can lyse resting microglia but spare activated microglia in an *in vitro* culture system¹⁰⁵. However, it remains unclear which NK cell subset executed this killing activity and how microglia were maintained within a resting state during this experiment.

Soluble factors that shape the NK cell phenotype in the CNS. Under pathophysiological conditions, an array of soluble factors is produced by microglia, astrocytes and neuronal cells (FIG. 2). Based on their effect on NK cells, these mediators can be categorized as inhibitory (such as TGF β , IL-10 and acetylcholine) or stimulatory (such as ATP, UTP, glutamate, prostaglandin E₂, H₂O₂, nitric oxide, IL-1 β , IL-6, TNF, epidermal growth factor (EGF), brain-derived neurotrophic factor (BDNF), neurotrophin 3, neurotrophin 4, IL-3, IL-12 and M-CSF). So, the sequence of soluble signals received by NK cells, the physical location of the cellular source of these factors and the balance between inhibitory and stimulatory signals that NK cells receive may all influence the functional status of NK cells in the inflamed CNS.

Role of CNS-specific NK cells in disease. During EAE in C57BL/6 mice, depletion of NK cells exacerbates neurological deficits^{9,11}. Similarly, CX₃CR1-deficient mice, in which homing of NK cells to the brain is severely impaired, develop fatal inflammatory lesions following EAE induction¹⁰. As the numbers of peripheral NK cells in these mice are comparable with those of wild-type mice, these results imply that a CNS-specific deficit in NK cells leads to aggressive inflammation and autoimmunity in the CNS (FIG. 2). Thus, there is strong evidence that CNS-specific NK cells control CNS inflammation during EAE in mice.

These findings might be relevant to the mode of action of daclizumab, a humanized antibody specific for the IL-2 receptor α -chain (CD25). Daclizumab, alone or in combination with IFN preparations, consistently reduces CNS lesions in patients with multiple sclerosis^{112–115}. One of the immunological effects of daclizumab is that it can expand CD56^{hi} NK cell populations in treated patients¹⁰⁷. In a human–mouse chimera model, it has been demonstrated that EAE can be ameliorated by transfusion of human NK cells¹¹. As administration of NK cells directly into the brain appeared to achieve enhanced clinical benefits compared with systemically administered cells¹¹, these results suggest that NK cells within the brain may have an anti-inflammatory role. Furthermore, there is some evidence to suggest that other drugs that are currently used for the treatment of multiple sclerosis, such as IFN preparations and glatiramer acetate (a random polymer of the four amino acids found in myelin basic protein; also known as copolymer 1 or Copaxone (Teva Pharmaceuticals)), may achieve their clinical efficacy partially by inducing NK cells^{116,117} via a mechanism similar to that of daclizumab.

In addition to their protective role in autoimmune disease, NK cells control CNS infections. In detail, NK cells provide protection against encephalitis and demyelination induced by Theiler's murine encephalitis virus¹¹⁸, reduce viral replication and disease following intracerebral infection by murine coronavirus¹⁰¹, and exacerbate immunopathology and accelerate mortality during Semliki Forest virus infection¹⁰².

In summary, there are no data on the presence of NK cells in the steady-state CNS, but NK cells have been shown to migrate to the inflamed CNS. Although the exact cellular and molecular interactions that shape NK cell phenotype and function in the CNS still need to be determined, it appears that activated NK cells have a protective role in the brain under the circumstances of ongoing inflammation, as they eliminate viral infection and inhibit the activation of autoimmune T cells through the killing of activated microglia.

Linking organ-specific NK cell features and disease

The major functions of NK cells in the liver, mucosal tissues, skin, pancreas and perhaps the CNS are to amplify the local immune response and combat infectious agents. In doing so, NK cells may kill infected or transformed cells. When this process becomes dysregulated, damage to neighbouring cells and autoimmune disease might ensue. Determinants for these divergent roles of NK cells, as exemplified here for a number of different organs, include the timing of immune responses, the different pathogens targeted by the NK cells and the NK cell-orchestrated immune responses. Furthermore, the studies discussed here raise the possibility that organ-specific factors shape NK cell phenotype and contribute to the organ-specific functions of these cells. The organ-specific features of NK cells appear to be dictated by a number of factors that are intrinsic to a particular organ, including its anatomical structure, accessibility to peripheral immune cells, and the unique microenvironment of cells and soluble factors within the organ. Additional determinants include inciting pathogens or autoantigens that induce inflammation.

Concluding remarks

We have discussed here the organ-specific features of NK cells and given examples of how these cells may serve to maintain normal health and contribute to the development and control of inflammation in various organ-specific diseases. There are many outstanding questions and future challenges in this area (BOX 2). Progress will be facilitated by novel approaches such as two-photon microscopy, which has been used to record the trafficking of several types of lymphocytes to reveal the cellular interactions within solid organs in physiological and pathological situations^{119–122}. The application of this and other techniques to studies of NK cells in solid organs should reveal valuable information on the physical localization of NK cells within specific anatomical structures, as well as interactions with resident cellular components. Imaging studies in live animals may yield insight into the behaviour of NK cells during the course of organ-specific inflammation.

Multiple sclerosis

An inflammatory and demyelinating disease of the central nervous system that is primarily diagnosed in young adults. Clinical presentations are heterogeneous depending on the severity and location of central nervous system lesions.

Box 2 | Outstanding questions on the organ-specific features of NK cells

- What determines natural killer (NK) cell recruitment to different organs, and how does the phenotype of NK cells change following recruitment to specific organs under different experimental conditions?
- Under what conditions do local NK cells become activated in different organs?
- Are specific features of NK cells within individual organs the cause or the consequence of inflammatory responses within that organ?
- How is the phenotype of NK cells linked to their particular functions within the inflamed organs?
- How can organ-intrinsic factors that boost or silence certain NK cell functions be harnessed to control infection or reduce inflammation?
- Do inflamed organs retain the recruited NK cells in an activated state, or do they allow for NK cell circulation back to other tissues and/or restoration of a resting NK cell phenotype?
- How are the local effects of NK cells affected by tissue-specific MHC class I expression? In addition to altering effector responses, how do the different levels of tissue MHC class I expression affect the functionality of local NK cells through education or licensing processes? How are these processes affected by local inflammatory responses and the influx of additional inflammatory cells that may alter tissue MHC class I expression levels?

High-throughput DNA and protein arrays, aided by laser capture microscopy to sample highly purified NK cells from localized areas of pathology, may aid in defining molecular signatures of NK cells within the inflamed organs. Improved access to human organs will also be paramount. Furthermore, improved ‘humanized’ mouse models that are capable of maintaining transplanted, functional human immune cells, including NK cells, should aid our understanding of human NK cells in mouse organs. Many mouse cytokine, chemokine and cellular (for example, Notch-induced) signalling pathways can trigger the expression of human NK cell receptors¹²³. Improving our

understanding of organ-specific NK cell functions through the use of these techniques will allow us to take advantage of organ-intrinsic factors for boosting specific NK cell functions to control infection or tumours, or to silence excessive effector functions to protect against organ-specific autoimmunity.

During evolution, distinct organs might have evolved different ways to recruit and influence the effector functions of NK cells. Once we understand these mechanisms, the next challenge will be to exploit this information to harness NK cells for combating infectious agents and tumours and for avoiding autoimmune and inflammatory diseases.

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Competing interests statement

The authors declare no competing financial interests.

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